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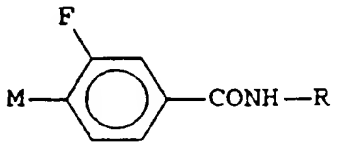
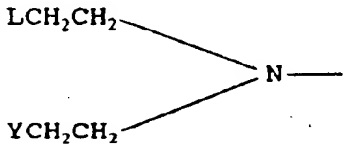
(54) Title: 4-AMINO-FLUOROBENZAMIDES AND THEIR USE AS CYTOTOXIC PRODRUGS




(A)




(A')

(57) Abstract

The invention provides a compound which is a 3-fluorobenzamide of formula (A) wherein R-NH is the residue of an α-amino acid R-$NH_2$ or oligopeptide R-$NH_2$, and M is a nitrogen mustard group of formula (A') wherein Y and L, which may be the same or different in a molecule, are leaving groups; or a pharmaceutically acceptable salt thereof. The compounds are useful as prodrugs for treating cancer.

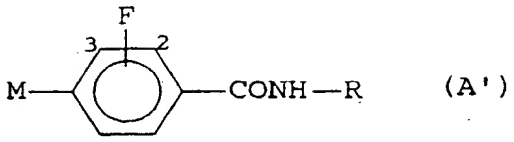
# 4-AMINO-FLUOROBENZAMIDES AND THEIR USE AS CYTOTOXIC PRODRUGS

This invention relates to prodrugs, their use in therapy and a process for their preparation.

Over the years, many cytotoxic compounds have been discovered which are of potential use in cancer chemotherapy. Nitrogen mustards from one important family of such cytotoxic compounds. The clinical use of cytotoxic compounds in general and nitrogen mustards in particular has been limited because of the poor selectivity in the cytotoxic effect between tumour cells and normal cells.

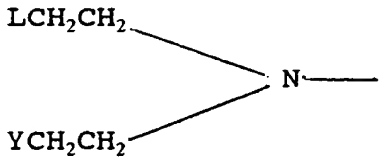
One approach to overcome this problem has involved the development of so-called prodrugs which are derivatives of the cytotoxic drug, often a relatively simple derivative, whose cytotoxic properties are considerably reduced compared to those of the parent drug. Numerous proposals have been made for the administration of such prodrugs to patients under regimes whereby the prodrug is only converted to the cytotoxic drug in the region of the intended site of action.

One particularly promising approach involves the conversion of the nitrogen mustard into a reaction product with an amino acid or oligopeptide to form a prodrug which can be ccnverted to the parent nitrogen mustard at the site of intended action under the influence of an enzyme. This approach can be put into practice by the utilization of an antibody/enzyme conjugate in association with a prodrug. The antibody/enzyme conjugate is one formed from an antibody to a tumour-associated antigen and an enzyme that will convert the prodrug to the cytotoxic drug. In clinical practice, the antibody/enzyme conjugate is first administered to the patient



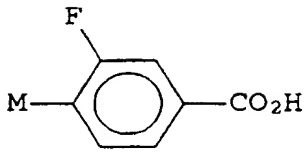

(A')

wherein R-NH is the residue of an α-amino acid R-$NH_2$ or oligopeptide R-$NH_2$, and M is a nitrogen mustard group of the formula




wherein Y and L, which may be the same or different in a molecule, are leaving groups; and pharmaceutically acceptable salts thereof. The F group may be at the 2- or 3- position relative to the -CONH-R group.

I have found that these compounds have surprising reactivities. Due to the strong inductive effect of fluorine, it would have been expected that a fluorine in the ring at position 2 or 3 would cause deactivation of the alkylating moiety, and that the inductive effect would be greater in the 3-position than in the 2-position. Thus, theoretically this would lead to the 3-fluoro compounds being less reactive than the 2-fluoro compounds. However, I found that although the 2-fluoro prodrugs and their corresponding drugs are deactivated as expected (i.e. less reactive than their non-fluorinated analogues), the 3-fluoro prodrugs and drugs are greatly activated (i.e. much more reactive than their non-fluorinated analogues). Further, all of the 3-fluoro but not all of the 2-fluoro prodrugs tested are good substrates for CPG 2.



(B)

wherein M is as defined above.

The prodrug is suitable for use in a method of treatment of the human or animal body by therapy, particularly a method of treatment of cancer. The invention includes a method of treating a human or animal suffering from cancer, which method comprises administering to the patient a prodrug of the invention. The cancer may be any disease in which there is neoplastic cell growth, including leukemias and solid tumours (e.g. colorectal and ovarian tumours).

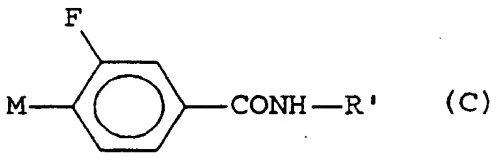
The prodrug may be selectively converted to the active drug by the enzyme component of an immunoglobulin/enzyme conjugate localised in the region of a tumour to be treated. Accordingly, the prodrug may be used in a method which comprises administering to a human or animal suffering from cancer pharmaceutically effective amounts of

(i) an immunoglobulin/enzyme conjugate in which the immunoglobulin is specific for a tumour-associated antigen, and the enzyme will cleave the amide bond between the residue of the α-amino acid R-$NH_2$ or oligopeptide R-$NH_2$ and the benzoic acid nitrogen mustard residue; and thereafter

(ii) the said prodrug.

Examples of suitable immunoglobulins and enzymes are given in WO-A-88/07378. The immunoglobulin may be an antibody

90/02729 and WO-A-91/03460. The process of the present invention comprises deprotecting a compound of the formula (C)




(C)

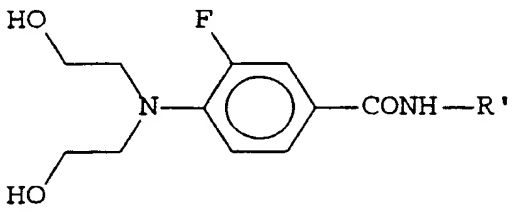
wherein M is as defined above, and R'-NH is the residue of an α-amino acid R'-$NH_2$ or oligopeptide R'-$NH_2$ containing at least one protected carboxylic acid group, and optionally converting the resulting compound of formula (A) as defined above into a pharmaceutically acceptable salt thereof. The compound of formula (C) is novel and forms part of the invention.

The at least one protected carboxylic acid group may, for example, be protected by an ethyl or tertiary butyl group. WO-A-88/07378 describes conventional methods of removing ethyl protecting groups which may be used in the present invention. In these methods, the ethyl protecting groups are removed by alkaline hydrolysis with aqueous sodium hydroxide followed by conversion of the resulting sodium salt into the free carboxylic acid using hydrochloric acid.

Preferably, the protecting groups are tertiary butyl. WO-A-90/02729 describes a suitable method of removing the tertiary butyl protecting groups. The tertiary butyl ester groups can be converted into free carboxylic acid groups by treatment with an acid, for example in a non-aqueous medium. Trifluoroacetic acid and formic acid are suitable acids. Removal of the tertiary butyl ester group can be carried out quite simply by maintaining the tertiary butyl ester in a

example, glutamic acid may be reacted with t-butylacetate. The compounds of formula (D) may be obtained from 3F, 4$NO_2$ toluene which are commercially available (e.g. from Aldrich Chemical Company Limited) by the method of Jackman et al., J. Med. Chem. (1990) 33, 3067-3071 and Marsham et al., ibid 3072-3078.

In a preferred method of producing the compound of formula (C), a compound of formula (E)



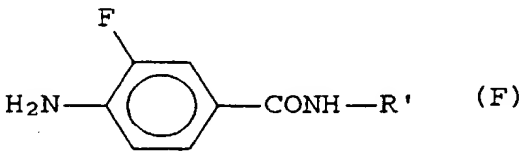

(E)

wherein R'-NH is as defined above, is reacted with a compound of formula

$$A\text{-}SO_2\text{-}B$$

wherein A is a methyl or 4-tolyl group, and B is a halogen (e.g. chlorine). The reaction is suitably carried out in an organic solvent, e.g. pyridine.

The compound of formula (E) is preferably prepared by reacting a compound of formula (F)




(F)

wherein R'-$NH_2$ is as defined above with ethylene oxide in a solvent, e.g. acetic acid.

The compound of formula (F) is preferably prepared by reducing a compound of formula (G)

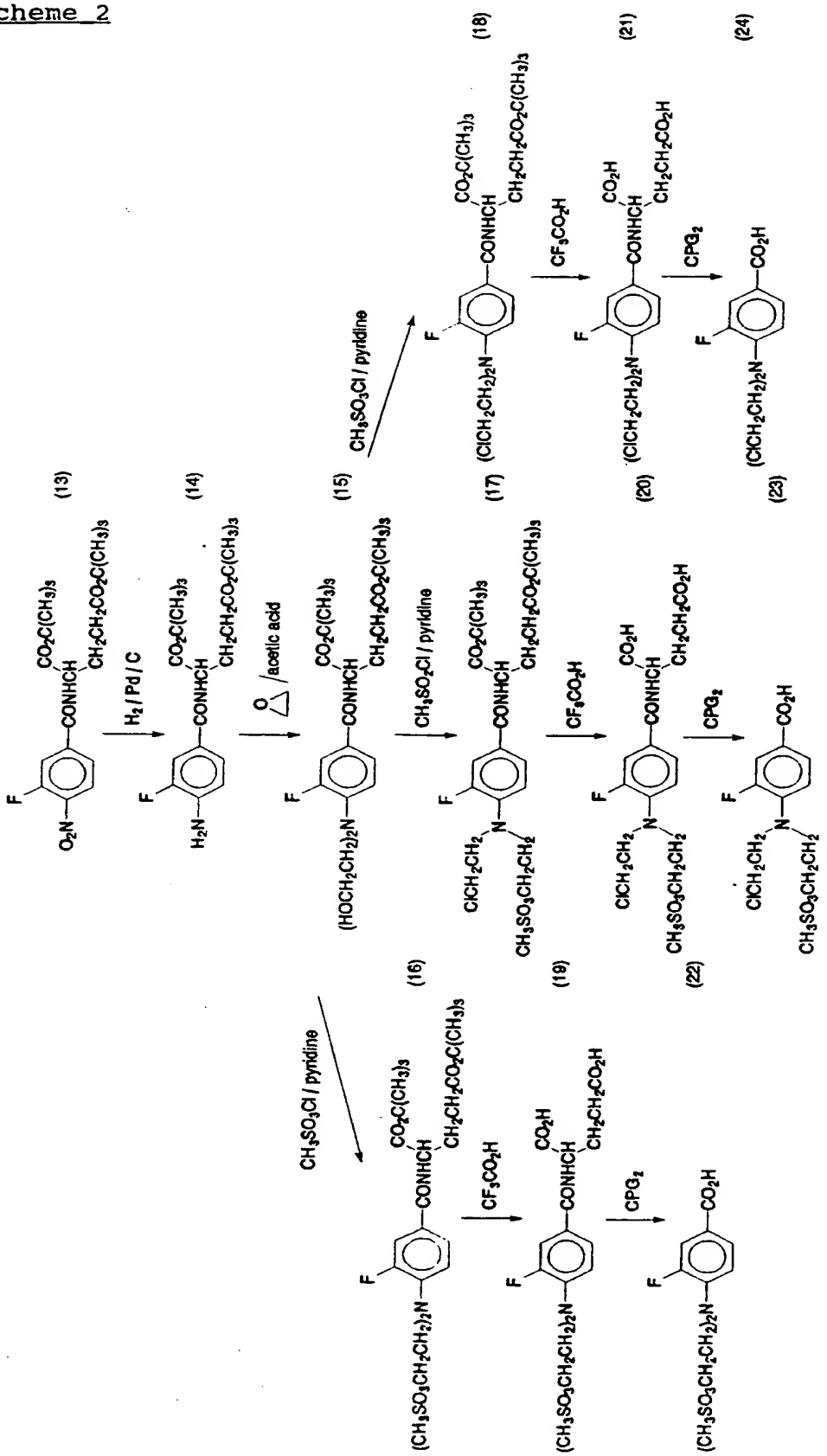
mustard residue.

The prodrug and immunoglobulin/enzyme conjugate will normally be administered parenterally, e.g. intravenously or intraperitoneally. Thus, the pharmaceutical composition of the invention is normally one which is suitable for parenteral (e.g. intravenous or intraperitoneal) administration. Such a composition conveniently contains the prodrug and isotonic saline or bicarbonate as diluent. The dose of the prodrug and conjugate will ultimately be at the discretion of the physician, who will take into account such factors as the age, weight and condition of the patient. Suitable doses of prodrug and conjugate are given in Bagshawe et al. Antibody, Immunoconjugates, and Radiopharmaceuticals (1991), 4, 915-922. A suitable dose of conjugate may be from 2000 to 200,000 enzyme units/$m^2$ (e.g. 20,000 enzyme units/$m^2$) and a suitable dose of prodrug may be from 20 to 2000 mg/$m^2$ (e.g. 200 mg/$m^2$).

In order to secure maximum concentration of the conjugate at the site of desired treatment, it is normally desirable to space apart administration of the two components by at least 4 hours. The exact regime will be influenced by various factors including the nature of the tumour to be targeted and the nature of the prodrug. A typical regime is to administer the conjugate at 0 h, galactosylated clearing antibody at 24 h, and prodrug at 48 h. If no clearing antibody is used, it would generally be longer than 48 h before the prodrug could be injected.

The following Examples serve to illustrate the invention. The following Reaction Schemes 1 and 2 summarise the processes of Examples 1 and 2 respectively.

Scheme 2



$_F$=13.93Hz, NH);

$^{19}$F NMR ($Me_2SO$-$d_6$) $\delta$-112.23 (ddd);

mass spectrum (FAB) m/z (397 [M+H$^+$], 100), 341 (M-t-Bu, 45);

Anal: $C_{20}H_{29}N_2O_5F$-0.5MeOH requires C-59.69, H-7.58, N-6.79, F-4.61, found C-59.84, H-7.48, N-7.02, F-4.79.

**Di-tert-butyl 2-fluoro,4-[Bis(2-hydroxyethyl)amino]benzoyl-L-glutamate (3)**

Amine (2) (1.6 g, 4.0 mmol) in HOAc (10 ml) was stirred with ethylene oxide (13.0 ml, 260 mmol) at room temperature for 112 h. The product was partitioned between $CH_2Cl_2$ and $H_2O$. The organic phase was separated, washed with $H_2O$, dried ($Na_2SO_4$), and evaporated to dryness. The crude oil was chromatographed on silica gel, eluting with EtOAc-$CH_2Cl_2$ to give an oil (3); yield (1.0 g, 49%).

$^1$N NMR ($Me_2SO$-$d_6$) $\delta$1.39 (s, 9H, t-Bu), 1.42 (s, 9H, t-Bu), 1.93 (m, 2H, $\underline{CH_2}CH_2CO_2$-t-Bu), 2.29 (t, 2H, J=7.69Hz, $CH_2\underline{CH_2}CO_2$-t-Bu), 3.46 (d, 4H, J=5.23Hz, $(HO\underline{CH_2}CH_2)_2$), 3.55 (t, 4H, J=4.80Hz, $(HOCH_2\underline{CH_2})_2$), 4.34 (m, 1H, CH), 4.75 (t, 2H, J=4.67Hz, $(OH)_2$), 6.50 (dd, 1H, $J_{H\text{-}3,F}$=17.09Hz, H-3), 6.57 (dd, 1H, $J_{H\text{-}5,H\text{-}6}$=8.97Hz, H-5), 7.33 (dd, 1H, $J_{H\text{-}6,F}$=9.1Hz, H-6), 7.69 (dd, 1H, $J_{H\text{-}N,H\text{-}C}$=7.11, $J_{H\text{-}N,F}$=14.07 Hz, NH);

$^{19}$F NMR ($Me_2SO$-$d_6$) $\delta$-111.03 (ddd);

mass spectrum (FAB) m/z (485[M+H$^+$],4), 226 (M-glut$Bu_2$, 100);

Anal: $C_{24}H_{37}N_2O_7F$-0.5EtOAc requires C-59.07, H-7.82, N-5.30, F-3.59, found C-59.23, H-7.71, N-5.20, F-3.32. (The presence of EtOAc noted in the elemental analysis was confirmed by NMR).

Eluting second was an oil, the 2-fluoro, (2-chloroethyl)[2-(mesyloxy)ethyl] derivative (5); yield (0.58 g, 34%);

$^1$H NMR ($Me_2SO$-$d_6$) $\delta$1.38 (s, 9H, t-Bu), 1.42 (s, 9H, t-Bu), 1.93 (m, 2H, $\underline{CH_2}CH_2CO_2$-t-Bu), 2.30 (t, 2H, J=7.82Hz, $CH_2\underline{CH_2}CO_2$-t-Bu), 3.15 (s, 3H, $\underline{CH_3}SO_3$), 3.77 (s, 4H, $Cl\underline{CH_2CH_2}$), 3.82 (t, 2H, J=5.18Hz, $CH_3SO_3CH_2\underline{CH_2}$), 4.32 (t, 3H, J=5.17Hz, $CH_3SO_3\underline{CH_2}CH_2$ & CH), 6.66 (m, 2H, H-3, H-5), 7.55 (dd, 1H, $J_{H\text{-}6,H\text{-}5}$=8.79, $J_{H\text{-}6,F}$=9.2Hz, H-6), 7.89 (dd, 1H, $J_{H\text{-}N,H\text{-}C}$=5.64, $J_{H\text{-}N,F}$=12.82Hz, NH);

$^{19}$F NMR ($Me_2SO_2$-$d_6$) $\delta$-110.45 (m);

mass spectrum (FAB) m/z (581 [M+M$^+$], 14), 322 (M-glut$Bu_2$, 100);

Anal: $C_{25}H_{38}N_2O_8FClS$ requires C-51.67, H-6.59, N-4.82, F-3.27, Cl-6.10, S-5.52, found C-51.92, H-6.53, N-4.82, F-3.16, Cl-6.06, S-5.48.

The fastest eluting, 2-fluoro, bis(2-chloroethyl) derivative was a solid (6); mp 104-106°C, yield (0.53 g, 34%);

$^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.38 (s, 9H, t-Bu), 1.42 (s, 9H, t-Bu), 1.96 (m, 2H, $\underline{CH_2}CH_2CO_2$-t-Bu), 2.29 (t, 2H, J=7.79Hz, $CH_2\underline{CH_2}CO_2$-t-Bu), 3.78 (dt, 8H, J=5.29Hz ($CI\underline{CH_2CH_2})_2$), 4.35 (m, 1H, CH), 6.65 (m, 2H, H-3, H-5), 7.55 (dd, 1H, $J_{H\text{-}6,H\text{-}5}$=9.1, $J_{H\text{-}6,H\text{-}5}$=9.4Hz, H-6), 7.88 (dd, 1H, $J_{H\text{-}N,H\text{-}C}$=5.53, $J_{H\text{-}N,F}$ =12.84Hz, NH)

$^{19}$F NMR ($Me_2SO$-$d_6$) $\delta$-110.55(ddd, $J_{F,H\text{-}N}$=11.26, $J_{F,H\text{-}3}$=14.07, $J_{F,H\text{-}6}$=16.32Hz);

mass spectrum (FAB) m/z (521 [M+H$^+$], 16), 262 (M-glut$Bu_2$, 100)

Anal: $C_{24}H_{35}N_2O_5Cl_2$ requires C-55.28, H-6.77, N-5.37, F-3.64, Cl-13.60, found C-55.43, H-6.82, N-5.39, F-3.62, Cl-13.91.

$^1$H NMR ($Me_2SO$-$d_6$) $\delta$2.02 (m, 2H, $\underline{CH_2}CH_2CO_2H$), 2.32 (t, 2H, J=7.53Hz, $CH_2\underline{CH_2}CO_2H$), 3.15 (s, 3H, $\underline{CH_3}SO_3$), 3.77 (s, 4H, $Cl\underline{CH_2}\underline{CH_2}$), 3.82 (t, 2H, J=5.11Hz, $CH_3SO_3CH_2\underline{CH_2}$), 4.32, (t, 2H, J=5.31Hz, $CH_3SO_3\underline{CH_2}CH_2$), 4.40 (q, 1H, J=4.54Hz, CH), 6.67 (m, H-3, H-5), 7.57 (dd, 1H, $J_{H\text{-}6,F}$=9.1, $J_{H\text{-}6,H\text{-}5}$=9.4Hz, H-6), 7.88 (dd, 1H, $J_{H\text{-}N,H\text{-}C}$=6.53, $J_{H\text{-}N,F}$=13.06Hz, NH);

$^{19}$F NMR ($Me_2SO$-$d_6$) $\delta$-110.35 (ddd, $J_{F,H\text{-}3}$=16.19Hz);

mass spectrum (FAB) m/z (469 [M+H$^+$],8), 322 (M-glu, 100);

Accurate mass Expected 469.0847 found +3.2 ppm;

Anal: $C_{17}H_{22}N_2O_8FClS$-0.26TFA-0.15EtOAc requires C-42.52, H-4.62, N-5.48, F-6.60, Cl-6.93, S-6.27, found C-42.12, H-4.68, N-5.13, F-6.20, Cl-6.67, S-6.0. (The presence of EtOAc and TFA, noted in the elemental analysis was confirmed by NMR).

This compound reacted positively with the Epstein spray reagent.

Compound (9); yield (0.17 g, 97%), 2-fluoro, 4-[bis(2-chloroethyl)amino]benzoyl-L-glutamic acid, was likewise obtained as an oil from (6);

$^1$H NMR ($Me_2SO$-$d_6$) $\delta$1.98 (m, 2H, $\underline{CH_2}CH_2CO_2H$), 2.33 (t, 2H, J=7.70Hz, $CH_2\underline{CH_2}CO_2H$), 3.78 (dt, 8H, $(Cl\underline{CH_2}\underline{CH_2})_2$), 4.41 (m, 1H, CH), 6.65 (m, 2H, H-3, H-5), 7.58 (dd, 1H, $J_{H\text{-}6,H\text{-}5}$=8.83, $J_{H\text{-}6,F}$=9.1Hz, H-6), 7.85 (dd, 1H, $J_{H\text{-}N,H\text{-}C}$=5.53, $J_{H\text{-}N,F}$=12.84Hz, NH);

$^{19}$F NMR ($Me_2SO$-$d_6$) $\delta$-110.43(ddd, $J_{F,H\text{-}3}$=15.27Hz);

mass spectrum (FAB) m/z (409[M+H$^+$]3),262 (M-glu, 100);

Accurate mass expected 409.0733 found +3.7 ppm;

Anal: $C_{16}H_{19}N_2O_5FCl_2$-0.40TFA requires C-44.36, H-4.30, N-6.16, F-9.19, Cl-15.58, found C-44.59, H-4.29, N-5.83, F-8.81, Cl-15.58. (The presence of TFA noted in the elemental analysis

(m, 2H, $\underline{CH_2}CH_2CO_2$-t-Bu), 2.31 (t, 2H, J=7.44Hz, $CH_2\underline{CH_2}CO_2$-t-Bu), 4.28 (m, 1H, CH), 5.71 (s, 2H, $NH_2$), 6.77 (dd, 1H, $J_{H\text{-}5,H\text{-}6}$=8.77, $J_{H\text{-}5,F}$=17.43Hz, H-5), 7.49 (dd, 1H, $J_{H\text{-}6,H\text{-}5}$=8.32Hz, H-6), 7.56 (dd, 1H, $J_{H\text{-}2,F}$=12.79Hz, H-2), 8.19 (d, 1H, J=7.55Hz, NH);

$^{19}F$ NMR ($Me_2SO$-$d_6$) $\delta$-135.56 (dd, $J_{F,H\text{-}2}$= 12.21, $J_{F,H\text{-}5}$=20.51 Hz);

mass spectrum (FAB) m/z 396 ([M+$H^+$],5} 138 (M-glu, 100);

Anal: $C_{20}H_{29}N_2O_5F$ requires C-60.59, H-7.37, N-7.07, F-4.79, found C-60.50, H-7.34, N-7.09, F-4.69.

**Di-tert-butyl 3-fluoro,4-[Bis(2-hydroxyethyl)amino]benzoyl-L-glutamate (15)**

Amine (14) (5.3 g, 13.4 mmol) in HOAc (30 ml) was stirred with ethylene oxide (60 ml, 1.2 mol) at room temperature for 336 h. The solvent was partitioned between EtOAc and $H_2O$. The organic phase was separated, washed with $H_2O$, dried ($Na_2SO_4$), and evaporated to dryness. The crude oil was chromatographed on silica gel, eluting with EtOAc-$CH_2Cl_2$ to give the pure oil (15); yield (3.3 g, 51%)

$^1H$ NMR ($Me_2SO$-$d_6$) $\delta$1.39 (s, 9H, t-Bu), 1.41 (s, 9H, t-Bu), 1.97 (m, 2H, $\underline{CH_2}CH_2CO_2$-t-Bu), 2.32 (t, 2H, J=7.42Hz, $CH_2\underline{CH_2}CO_2$-t-Bu), 3.43 (t, 4H, J=5.93Hz, $(HO\underline{CH_2}CH_2)_2$), 3.54 (d, 4H, J=5.46Hz, $(HOCH_2\underline{CH_2})_2$), 4.31 (m, 1H, CH), 4.67 (s, 2H, $(OH)_2$), 6.99, (dd, 1H, $J_{H\text{-}5,H\text{-}6}$=8.86, $J_{H\text{-}5,F}$= 17.84Hz H-5), 7.60 (dd, 2H, $J_{H\text{-}6,H\text{-}5}$=9.56, $J_{H\text{-}2,F}$=14.26Hz H-6, H-2) 8.30 (d, 1H, J=7.48Hz, NH);

$^{19}F$ NMR ($Me_2SO$-$d_6$) $\delta$-124.31 (dd, $J_{F,H\text{-}2}$=11.63, $J_{F,H\text{-}5}$=17.08Hz);

mass spectrum (FAB) m/z (485 [M+$H^+$], 22), 226 (M-glutBu, 100);

Anal: $C_{24}H_{37}N_2O_7F$-1.1EtOAc requires C-58.66, H-7.94, N-4.82, F-3.27, found C-58.31, H-7.83, N-5.18, F-3.49. (The presence of EtOAc noted in the elemental analysis was confirmed by NMR).

Eluting second was the 3-fluoro, (2-chloroethyl)[2-(mesyloxy)ethyl] derivative, as the oil (17); yield (0.29 g, 37%);

$^1$H NMR ($Me_2SO$-$d_6$) δ1.39 (s, 9H, t-Bu), 1.41 (s, 9H, t-Bu), 1.99 (m, 2H, $\underline{CH_2}CH_2CO_2$-t-Bu), 2.32 (t, 2H, J=7.37Hz, $CH_2\underline{CH_2}CO_2$-t-Bu), 3.12 (s, 3H, $CH_3SO_3$), 3.71 (s, 6H, $Cl\underline{CH_2CH_2}$ + $CH_3SO_3CH_2\underline{CH_2}$), 4.30 (t, 3H, J=5.29Hz, $CH_3SO_3\underline{CH_2}CH_2$ + CH), 7.13 (dd, 1H, $J_{H\text{-}5,H\text{-}6}$=8.81, $J_{H\text{-}5,F}$=9.0Hz, H-5), 7.66 (dd, 2H, $J_{H\text{-}2,F}$=14.58Hz, H-2, H-6), 8.41 (d, 1H, J=7.54Hz, NH);

$^{19}$F NMR ($Me_2SO$-$d_6$) δ-123.40 (m);

mass spectrum (FAB) m/z (581 [M+H$^+$]30),322 (M-glut$Bu_2$, 100);

Anal: $C_{25}H_{38}N_2O_8FCIS$ requires C-51.67, H-6.59, N-4.82, F-3.27, Cl-6.10 S-5.52, found C-51.29, H-6.60, N-4.56, F-3.18, Cl-5.74, S-5.29.

The fastest eluting, 3-fluoro, bis(2-chloroethyl) derivative was the solid (18), mp 100-103°C; yield (0.11 g, 15%);

$^1$H NMR ($Me_2SO$-$d_6$) δ1.39 (s, 9H, t-Bu), 1.41 (s, 9H, t-Bu), 2.01 (m, 2H, $\underline{CH_2}CH_2CO_2$-t-Bu), 2.33 (t, 2H, J=7.34Hz, $CH_2\underline{CH_2}CO_2$-t-Bu), 3.72 (s, 8H, $(Cl\underline{CH_2CH_2})_2$), 4,32, (m, 1H, CH), 7.11 (dd, 1H, $J_{H\text{-}5,H\text{-}6}$=8.86, $J_{H\text{-}5,F}$=9.1Hz, H-5), 7.65 (m, 2H, H-2, H-6), 8.40 (d, 1H, J=7.35Hz, NH);

$^{19}$F NMR ($Me_2SO$-$d_6$) δ-123.83 (dd, $J_{F,H\text{-}2}$=14.8 Hz);

mass spectrum (FAB) m/z (521 (M+H$^+$], 19), 262 (M-glut$Bu_2$, 100);

Anal: $C_{24}H_{35}N_2O_5Cl_2$-0.5$H_2O$ requires C-54.34, H-6.84, N-5.28, F-3.58, Cl-13.37, found C-54.71, H-6.61, N-5.31, F-3.64, Cl-13.54.

+ $CH_3SO_3CH_2\underline{CH_2}$), 4.31 (t, 2H, J=5.40Hz, $CH_3SO_3\underline{CH_2}CH_2$), 4.39 (m, 1H, CH), 7.15 (dd, 1H, $J_{H-5,H-6}$=8.81, $J_{H-5,F}$=18.24Hz, H-5), 7.68 (dd, 2H, $J_{H-2,F}$=14.75Hz, H-2, H-6), 8.45 (d, 1H, J=7.64Hz, NH);

$^{19}F$ NMR ($Me_2SO-d_6$) $\delta$-123.19 (dd, $J_{F,H-2}$=11.46, $J_{F,H-5}$=14.12Hz);

mass spectrum (FAB) m/z (469 [M+H$^+$], 10), 322 (M-glu, 100);

Accurate mass expected 469.0847 found +4.9ppm;

Anal: $C_{17}H_{22}N_2O_8FCIS$-0.20TFA-0.21EtOAc requires C-42.94, H-4.72, N-5.49, F-5.96, Cl-6.95, S-6.28, found C-43.34, H-4.79, N-5.16, F-5.95, Cl-6.82, S-5.89. (The presence of EtOAc and TFA noted in the elemental analysis was confirmed by NMR).

This compound reacted positively with the Epstein spray reagent.

Compound (21); yield (0.05 g, 97%), 3-fluoro, 4-[bis(2-chloroethyl)amino] benzoyl-L-glutamic acid, was likewise obtained as an oil from (18):

$^1H$ NMR ($Me_2SO-d_6$) $\delta$2.00 (m, 2H, $\underline{CH_2}CH_2CO_2H$), 2.35 (t, 2H, J=7.48Hz, $CH_2\underline{CH_2}CO_2H$), 3.73 (s, 8H, $(Cl\underline{CH_2CH_2})_2$), 4.41 (m, 1H, CH), 7.12 (dd, 1H, $J_{H-5,H-6}$=8.78, $J_{H-5,F}$=18.17Hz, H-5), 7.67 (dd, 2H, $J_{H-2,F}$=15.39Hz, H-2, H-6), 8.42 (d, 1H, J=7.22Hz, NH);

$^{19}F$ NMR ($Me_2SO-d_6$) $\delta$-123.65(dd);

mass spectrum (FAB) m/z (409 [M+H$^+$],48), 262 (M-glu, 100);

Accurate mass expected 409.0733 found -0.7ppm;

Anal: $C_{16}H_{19}N_2O_5FCl_2$-0.18TFA-0.2EtOAc requires C-46.07, H-4.68, N-6.26, F-6.54, Cl-15.85, found C-46.29, H-4.80, N-5.99, F-6.29, Cl-15.99. (The presence of EtOAc and TFA noted in the elemental analysis were confirmed by NMR).

This compound reacted positively with the Epstein spray reagent.

**TABLE 1**

**Kinetics of Prodrugs as substrates for CPG2**

| PRODRUG | $K_m$/μmol | $k_{cat}$/$s^{-1}$ |
|---|---|---|
| 7 | very poor substrate | |
| 8 | 11 | 213 |
| 9 | 15 | 462 |
| 19 | 17 | 565 |
| 20 | 6 | 614 |
| 21 | 10 | 1028 |

**EXAMPLE 4: REACTIVITY OF THE PRODRUGS AND ACTIVE DRUGS**

The chemical half lives of the prodrugs and the active drugs were measured in order to determine their relative reactivities.

The half lives were measured in a pH stat, by titrating against NaOH, according to Springer et al, Anticancer Drug Design (1991) 6 467-479. The results are shown in Table 2. All three 2-fluoro prodrugs (7, 8, and 9) and their corresponding active drugs (10, 11 and 12) were deactivated. The chemical half lives of the 2-fluoro prodrugs were too long to be measured in a pH stat. In contrast, the 3-fluoro prodrugs (19, 20 and 21) and the corresponding drugs (22, 23 and 24) were activated compared to the corresponding non-fluorinated analogues and 2-fluoro analogues.

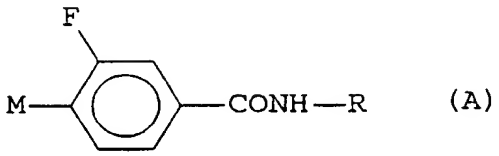
**EXAMPLE 5: CYTOTOXICITY OF THE PRODRUGS WITH AND WITHOUT CPG2 IN A COLORECTAL CELL LINE**

The 2- and 3-fluoro prodrugs 7-9 and 19-21, and the non-fluorinated prodrug 26 were tested for prodrug activity by measruing their cytotoxicity with and without CPG2 in the colorectal cell line LS174T for 1 h (Tom et al (1976) In Vitro 12, 180-181). The corresponding active drugs 10-12, 22-24 and 29 respectively were screened under the same conditions.

The results are shown in Table 3. All the 3-fluoro prodrugs 19-21 showed substantial prodrug activity as did the non-fluorinated prodrug 26. In each case the prodrug was completely non-cytotoxic even at 800 μM and conversion to its corresponding drug by CPG2 led to increased cytotoxicity. The cytotoxicity of each of the active drugs 22-24 and 29 alone was not significantly different from that of its prodrug + CPG2 (19-21 and 26) respectively. Although all the 2-fluoro prodrugs alone were non-toxic, none exhibited prodrug activity since they were not converted to a cytotoxic species in the prodrug + CPG2 tests. These data were in good argument with the cytotoxicity experiments using the 2-fluoro active drugs.

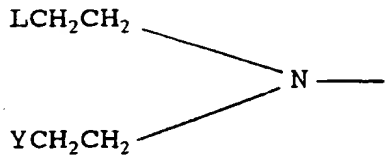
## CLAIMS

1. A compound which is a 3-fluorobenzamide of the formula (A)




(A)

wherein R-NH is the residue of an α-amino acid R-$NH_2$ or oligopeptide R-$NH_2$, and M is a nitrogen mustard group of the formula




wherein Y and L, which may be the same or different in a molecule, are leaving groups; or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein Y and L, which may be the same or different in a molecule, are selected from halo, mesyloxy and 4-tosyloxy.

3. A compound according to claim 2 wherein Y and L are both mesyloxy, Y and L are both chloro, or Y is mesyloxy and L is chloro.

4. A compound according to any one of the preceding claims wherein the amino acid R-$NH_2$ is glutamic acid or aspartic acid.

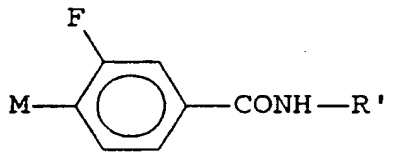
5. A compound according to any one of the preceding claims wherein the amino acid R-$NH_2$ is an L-amino acid.

6. A compound according to claim 1 which is

the α-amino acid R-$NH_2$ or oligopeptide R-$NH_2$ and the benzoic acid nitrogen mustard residue; and thereafter

(ii) the said compound or composition.

12. A process for producing a compound as claimed in any one of claims 1 to 6 and 9 to 11, which process comprises deprotecting a compound of the formula (C)

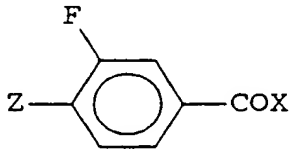



(C)

wherein M is as defined in claim 1, 2 or 3, and R'-NH is the residue of an α-amino acid R'-$NH_2$ or oligopeptide R'-$NH_2$ containing at least one protected carboxylic acid group, and optionally converting the resulting compound of formula (A) as defined in claim 1 into a pharmaceutically acceptable salt thereof.

13. A process according to claim 12 wherein the at least one protected carboxylic acid group is protected by an ethyl or a tertiary butyl group.

14. A process according to claim 12 or 13 wherein the compound of formula (C) is obtained by reacting a compound of formula (D)




(D)

wherein X is hydroxy or halo and Z is a group capable of being converted to a nitrogen mustard group M as defined in claim 1,

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/00941

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07C237/36 C07C229/60 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | J. MED. CHEM. (JMCMAR,00222623);90; VOL.33 (2); PP.677-81 CHARING CROSS HOSP.;DEP. MED. ONCOL.; LONDON; W6 8RF; UK (GB) Springer C J et al 'Novel prodrugs which are activated to cytotoxic alkylating agents by carboxypeptidase G2' cited in the application see the whole document --- | 1 |
| A | WO,A,88 07378 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.;UK ) 6 October 1988 cited in the application see claims 15-18 ----- | 1 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 1 August 1994 | 12. 08. 94 |

| Name and mailing address of the ISA | Authorized officer |
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 94/00941

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| WO-A-8807378 | 06-10-88 | DE-D- | 3889340 | 01-06-94 |
| | | EP-A- | 0408546 | 23-01-91 |

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